

Figure 1 : Isolement de souche monocaryotique déficiente pour l'activité laccase

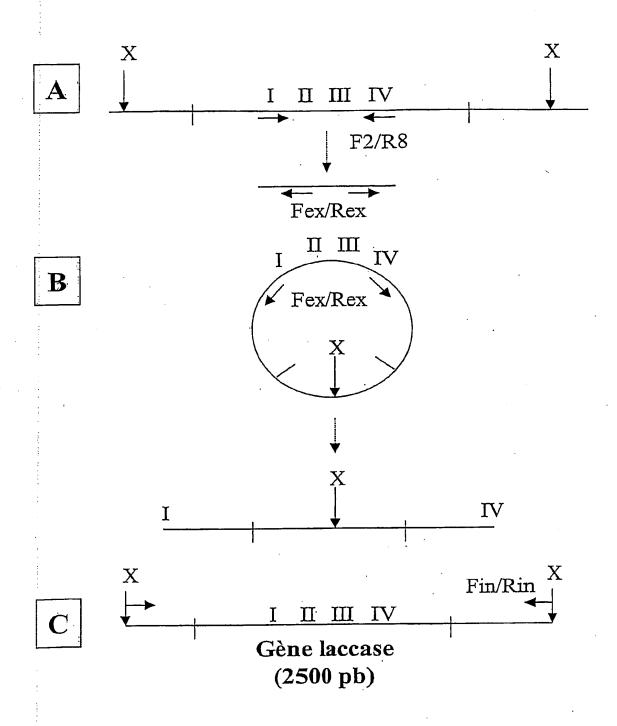
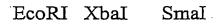


Figure 2 : Isolement du gène codant pour la laccase de *Pycnoporus* cinnabarinus laccase



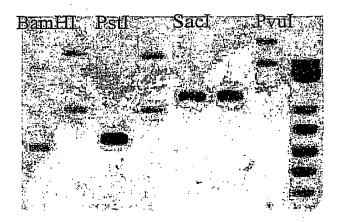


Figure 3 : Etude en Southern blot du gène codant pour la laccase de *Pynoporus cinnabarinus*

CTGCAGACATCTGGAGCGCCTGTCTTTCCCCTAG <u>TATAAA</u> TGATGTCTGTCCGCAGGTCCTTGAAGACCGCTCGAGTCCCACTTGAGTTTTAGGTAGG		1.00
CTGTCCAAACCCCTCTTTCTGATCATGTCGAGGTTCCAGTCCCTCTTCTTCTTCGTCCTCGTCTCACCGCTGTGGCCAACGCAGCCATAGGGC M S R F Q S L F F F V L V S L T A V A N A A I G P	25	200
CTGTGGCGGACCTGACCCTTACCAATGCCCAGGTCAGCCCCGATGGCTTCGCTGGGGAGGCCGTCGTGGTGAACGGTATCACCCCTGCCCCTCTCATCAC		300
V A D L T L T N A Q V S P D G F A R E A V V V N G I T P A P L I T AGGCAATAAGGtatgtatatatgctcgtccctcagagctacatacatctgatccacaatcgtttagGGCGATCGATCCAGCTCAATGTCATCGACCAG G N K G D R F Q L N V I D Q	58 72	400
F2		
TTGACAAATCATACCATGTTGAAAACATCTAGTATTgtaagggttcagtttttcccgactaccatgttattgaccatcaccactcgtag CATTGGCACGG L T N H T M L K T S S I (I)	88	500
CTTCTTCCAGCAAGGCACGAACTGGGCCGATGGTCCCGCGTTCGTGAACCAGTGTCCCATCGCTTCGGGCCACTCGTTCTTGTATGACTTTCAAGTTCCC FFQQGFNWADGPAFVNQCPIASGHSFLYDFQVP	121	600
(I) GACCAAGCAGgtacgaattccgtacacgtttcsttgcgtcgcaactaaacctcctcttactagGGACTTTCTGGTACCATAGCCATCTCTCCACGCAAAA		700
DQAG TFWYHSHLSTQY (II)	137	
CTGCGATGGTTTGAGGGGGCCTTTCGTCGTCTACGACCCCAACGATCCTCACGCTAGCCTTATGACATTGACATTGATAACGGtgagcagatcatggtatcgcaa	163	800
tattgegteeacttatgetteetggeateeagacGACACTGTCATTACGCTGGCTGATTGGTATCACGTTGCTGCCAAGCTCGGACCTCGCTTCCCgtac D T V I T L A D W Y H V A A K L G P R F P		900
gtgtcaaatgtctacgagagatctcacatatacgactagactcacttcgctgattacagATTTGGTTCGATCCGATTCAACCCTTATCAATGGACTTGGTCGAA	184	1000
CCACTGGCATAGCACCGTCCGACTTGGCAGTTATCAAGGTCACGCAGGCCAAGCGgtagtatggatggtcatcactggcacattggctctgatacattgc	198	1100
T G I A P S D L A V I R V T Q G K R CLUGLICCECAGCATACCATACATCACCATACACACACACACACACACA	216	1200
	245	
	268	1300
CAGACATICETAGATACCCCTCCCTTCCGAAACCCTGCTTGGATGCTAGCCAGGTT L D A 3 Q P V D N Y W I R A N P A F G N T G F	291	1400
TTGCTGGTGGAATCAATTCTGCCATCCTGCGTTATGATGGCGCACCCGAGATCGAGCCTACGTCTGTCCAGACTACCTAC	324	1500
CGACTTGGATCCTTCTCGCCTATGCCTGTGgtacgtgtctcaaagaacctcgatcactaagtgcatgtcaactcatatggtgcatgacatgtcaactcatgtgcatgacat		1600
CCCGAGCCCGGAGGTGTCGACAAGCCTCTGAACTTGGTCTTCAACTTCytgagtactggcgcttccgtagcacacgttcgaacaaagcctgataccat	337	1700
g eag aacgecaccaacttcttcatcaacgaccacacctttgtcccgcctctgtcccagtcttgctacaaatcctcagtgggggccaggcgctcag gac	353	1800
CTGGTCCCGGAGGGCAGCGTGTTCGTTCTTCCCAGCAACTCGTCCATTGAGATATCCTTCCCTGCCACTGCCAATGCCCCTGGATTCCCCCATCCGTTCC	385	1900
LVPEGSVFVLPSNSSIEISFPATANAPGFP <u>PPE</u>	419	
ACTTGCACGGTgtacgtctgccttcccctcgtctaaaggcggagtcgatatctgactcccatcacagCACGCCTTCGCTGTCCTCCCGAGCCGCC GGGAGC LRG BAFAVVRSAG BAFAVVRSAG GGAGC	433	2000
(III) AGCGTCTACAACTACGACAACCCGATCITCCGCGACGTCGTCAGCACCGGCCAGCCCGGCGACAA ACGCAACGATTCGCTTCGAGACCAATAACCCAGGCC		2100
SVYNYDNPIFRDVVSTGQPGDNVTIRFETNNPGP R8	4 67	
	500	2200
(IV) (IV) (IV) TCAGGCGTGGTCGGACTTGTGCCCCATCTATGATGCACTTGACCCCAGCGACCTCTGAGCGGGATTGTTACTGTGACCTGGT GTGGGGGGAACATGTCGA		2300
Q A W S D L C P I Y D A L D P S D L . GGGCTTTCATCGATCAGGGACTTTCAAGGTTGGCATAATATACCTCACGGCCTGGATGACTCGGACAGCGTGTGGGCGTGTAACTCTGCTTGATGT	518	2400
TGAAAAAAGGATTTTATGTAGAACAATTTATGAGCAATCAGCAATCAAT		2500
TGCGGGAAATGGGTCCATGATACACATCATTGAGCTCTCAATACCAAGAAGGATTACCCATGTCAATACCCAAGATCATGTCTTCGCTGTCCGCAATGG		2600
TCTCATGTTGCGTTGAGCAGATCGCAGTACGTTGAAAAGCGATTAGTAT TACATGCAACATGCAACATTTGGAAGGGGGCATGCAGAGGTTCAGCTCGCG		2700
*TCAGTCGGCCAAGTAGCGACCTTTGCCGCACTGCTGTTAACCTGAACGTATGCTTCAGAACTCCGTCGGTATCGAGAGCGATCGTGTACGTTCCGGGAT		2800
AGATCCATTGATCCCCGCTCTGGTCGGCGCGTGCGATGGCCCCGAGCGTCACCGGCAGCTTCGCGATCGCGCTTTTCCTAGGGGGCGAGGCCGTGTACCCG	:	2900
CGTGTACGAGACGAGCTGCTTGTTCGGGTGGGGCGAAGGCCCCGAAGGAGCCACTCACGAAGAGCAATGCGACGTAATCCGAGGTAGCCTTGCCCGTGTTA	3	9000
GTCACACGCACGGAGAACGTGTCGAGCGGCGCGAGGTCGAGGAAGGCGGCGCTCTTCTGACCGCGCTGTACGAGGTCGGAAATCGAATACGTCGATGGCG	;	3100
GTCCTCCAAAGTCCGTGACGTTGGTCGCATCGGCCGCCCGC	3	200
GACCGGCGTGCCGGTGTACCACTTGTATGTACGCCCCGGGTTCGACGCGCTTGGGCGAAGGGT CATGTCAGTCATCAGCAACCTGATCAGCGTAGATGGCT	:	300
GGGTATTGGGTGATGGGCÄGGCGTCCTGCAG	3	331

Figure 4 : Séquence du gène codant pour la laccase de *Pycnoporus cinnabarinus*

AGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCAGGGCAGCGACGTA CCGGCCGGCGTGCGCCATTGAGGTACATGAGCGGGGGGGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCG CGGCGATGAAACGTTTCCCACCATTGGGAAGAAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGC GGCGTCGCGCCTTTGCCGÇAGAGGCCGGCGGCGACATGCACAGCGAAGGTCCGTTGCGGATGGGAAGCAGG CAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGGAGCGTAGGCGCCCTCCCATAAGGCGGCAAGCATC ATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACC ATCGGCATCCTCAGCCTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGC TTCTTGGATGATCATGTCGTAACTTTTTCTGACCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGT ATGCCTCCCATTCATAAACGATAACCCCTTCCTTCAGGTTGGTCATCTCCATAGAGCGGCACGCTCTCAAGG CCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCATGGGATC ACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGT TCAGTCACCACATGGCAAAAAAGCTGCACCATACTCTTTATGGTGAGTTGTTCGTGAGTGGTATACAGTCAT TCATGAGGGAATGCCCACCGGATAGGGTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGT CCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAAACAGCGTTGACTGTGAATCCCTGGTGTGT GTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTGCGAGCGGCACAG GCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGG AGCGAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGAT TGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAAGTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGT CTCGGAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAATGCGCACACACGGAGGCTTTA CATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCT CTCATGACGTCCGCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATGGTAGTGGA GTCAGCCTGGCCAGTGCGTAGTCCCGTCTCTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTGC TTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTGCACGCCCAGAGGTATTGGGCTCGTCA GATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCTATAGCCCTT CTTCGCGCGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTAC CGAACAATTGACTTACCGACATCCTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCC GCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGACGGCCGCTCATCAGGACCAGACCAGTCTCAAT GTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTGGTGCACCGTCGCTTAC GATCATG

Figure 5 : Séquence de la séquence promotrice du gène codant pour la laccase de *Pycnoporus cinnabarinus* (jusqu'à l'ATG codant pour la méthionine de la laccase)

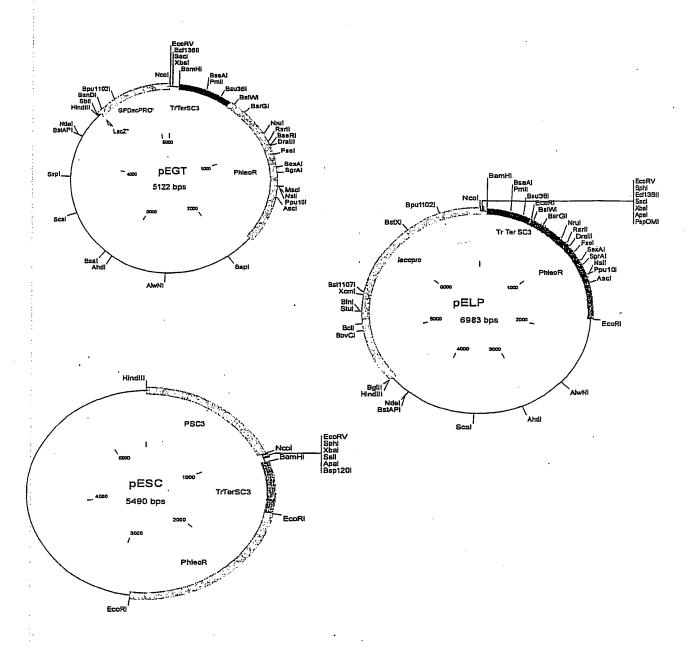


Figure 6 : Carte physique des trois vecteurs d'expression utilisés pour la production de la laccase chez *Pycnoporus cinnabarinus*

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCGCCTTAAGACGCGTGGATCCGCAGGTGAAC GCGCCTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGT TTCTCTATCCGCTCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAG GGGGGTACAAAAGGAGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCT CAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATC GTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACG AGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTTCACGAAGCTCTTGCTGATCGCGATGCCG GGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCGG CTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCAC CCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGC CCGGAACGGCACTGGTCAACTTGGCCATGCATGGTGATGGGCATTATGTGTGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATG CCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGTCCTTCTCGCACC TGGGCTGCGACCCTGTCTACCTCTCAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAGGCTCTCGCGC CACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGGGAATTCGT AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAG CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCT GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACG AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCC TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCCACTGGTAACAGGATTAGCAGA GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGTTT GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAA AACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAATGAAGTTTTAAATCAA TCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTC ATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG TGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATT GGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGA TCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAATGCCGCAAAAAAGGGAATAAGGGCGAC ACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCA TGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGC AGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGG TGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTA AGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTA CGCCAGCTGGCGAAAGGGGGATGTGCTAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC GCGGGCCCCGCGCCCCCTCGGGCGAGCGGTGTATCTACGAACGGAACTGGGAGGCGACTCGGAAGAGTTTGGTTAGAAAGGG GAACACCATCGCGGACGGCCCAGTGCTCTGGDCAGCTGAGCGTGCATTGTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC GGGATCGGAGGGTGGCGCGAGAGCCTCTTCGGTGTGAGATTAGTAACTGTACTGCGAAGCCGCGGAGGGGTTAGGATGAGAGGTAG ACAGGGTCGCAGCCCAGGTGCGAGGACTGCGAAGGACTGTTCTTCGACCGCGCACCTGCAATTGCGCGCATGGATAGAATAGA GCCGCCGATGTGTTCATCCCGTTTTGTCAGTATCGATCGGATCTTTCGGGCGTGGGTATAAAAGCGCGCCGCCGCCGTCTCCCT CTTTCTCCAGCACTCCCATCCAGAGCACTTCCCTCTCCCATCGCATCCCATCACACAATAATGCCCATCAC

Figure 7: Séquence nucléotidique du vecteur pEGT, contenant le promoteur du gène gpd (4480-5122), un marqueur de résistance à la phléomycine (507-1822) et le terminateur du gène sc3 (71-507).

AGCTTCTCCGGCCCGAATCGAACGGCAGGATGTGTGGGCGTGTCCAATATTGCCATGAAAATCTGTCAGAAGTGAGCCCTCTCGTCAC $\tt CCTGTACAGCTTCGCTGAGTTGAAAAGCAGGGTTCATCTTGGGCTCACTGATGCACTGAGCTCGACCGGAGAACTAAATGACCAGCCGG$ AGTGTTCACTAACTTAACGCCGGGTATTCAGGGCAGCTTCTCTATGTTGCGCCTACGACGTAGATCACCGCCCATGAACGGGGGAAACG GGGAGGGGTGCGTTTGGTACGTCTTTACGTCTGGCTATGTTGTATTGACCAGCGTCTGCAGAAGATGGGCACGACGATGCCCCGAGCCG AGGGGCTTAGATGGAGAGTGACACGTCTGAGCTCCCCAACACGCCTTCGCCGAGGGTGCGTCTCCGCGGGACATTCACCTCAGTTCATTG TTCTGACCTGCCTAATTGTATAGACCGGCCAACAACCTTGCTGACGCCCATCATAACAGTGCCCTGCACAGAGCCTTCCCACTCAGTCGG CGCCTCCCTCAATCAATCCCACTAACTCGCCGGCTCTGCCCCTTCGCCGCTCGACACGTCGCTTGGAAGAGCCCGGGCACGGGCGTCCGC AACGCGCGGGAAGAAATAATTTACGGGAGCCTCCCCAGGTATAAAAGCCCCTCACCCGCTCACTCTTTCTCCAGTCGAACACCCCAGT TCÁACTACCCAGCCCTTCCTTCCTTCGCTATCCTTCYTTACAACCTGCTCGCCATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGAC GGGCCCGGTACCGCGCCCCTTAAGACGCGTGGATCCGCAGGTGAACGCGCCTATCGGTGGGATATTCGGGCGACGGGACCCTCGGC AATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAG TTCGCCACGCAACAACGCCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGTGGACTAAAGCACTCCACTTTACGGCGAGGATGC CAGTCTACGTCATGAATGAAGCCTCAGGTCCCGAAGTAAGGGGGGTACAAAAGGAGGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCA CATACATGCACGACTTCAAAACATCCATTCTATCATTITGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCCTTTCTTCTT TTÄCTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAG AGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTT CACGAAGCTCTTGCTGATCGCGATGCCGGGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTC GCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGT CCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAG GCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGG GAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACAACATACGAGCCGGAAGCATAAAGTG TAAAGCCTGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA GCTGCATTAATGAATCGGCCAACGCGGGGAGAGGGGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTTCCATAGGCTCCGCCCCCTGACGAGC ATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTG CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTA ACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT CGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTT TTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTT .GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTTG GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTC CTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGT AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAAT ACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAA6GATCTTAC CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAA AÁACAGGAAGGCAAAATGCCGCAAAAAAGGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTCCTTTTTCAATATTATTGA AGCATTTATCAGGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCC CGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGC GCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGAC CATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGA AGGCCGATCGCTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCA

Figure 8 : Séquence nucléotidique du vecteur pESC, contenant le promoteur du gène sc3 (1-1033), un marqueur de résistance à la phléomycine (1540-2855) et le terminateur du gène sc3 (1104-1540)

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCGCCTTAAGACGCGTGGATCCGCAGGTGAACGCGC CTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGT TCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGT GTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACG CCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATCATTTTTGGGATCGTACAATTATTAGA CATGTTGTACAACGTTACATTCCTTTCTTTTACTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTT AGAGCGCCCGTCTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCC ACCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCAGTCCTGCTCCTCGGCCACGAAGTGCACGCAGTTG GACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGG CCGTCGGGCGCCACCACCAGCCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGA AGAACAGTCCTTCGCAGTCCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTA ATCTCACACCGAAGAGGCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTC ARATCAGATCCCCGGGAATTCGTAATCATGGTCATAGCTGTTTTCCTGTGTGAAATTGTTATCCGCTCACAAATTCCACACAACATACGAGCC AACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTG CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGGTTCAGCCCGACCGCTGCGCCTT ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCAAG TTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCTTTGG TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAA GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACA GGÅAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATT TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAG TOCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGG TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA GGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG TGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGT GCGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGGCCAGTGCCAAGCTTAGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCA CGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCGCGGCGATGAAACGTTTCCCACCA TTGGGAAGAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGCGGCGTCGCGCCTTTGCCGCAGAGGCCGGCGGCGACATGCA

Figure 9: Séquence nucléotidique du vecteur pELP, contenant le promoteur du gène laccase (4457-6983), un marqueur de résistance à la phléomycine (507-1822) et le terminateur du gène sc3 (71-507) (suite de la séquence, page suivante)

TAAGGCGGCAAGCATCATGATGCTCTCCGATTCGGGAAGCCTGGTGCQATGCTGGAGAGACTCTCTCCGAGAGACCCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACCATCGGCATCCTCAGC CATAGAGCGGCACGCTCTCAAGGCCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCAT GGGATCACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGTTCAGTCACCA GTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGTCCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAA ACAGCGTTGACTGTGAATCCCTGGTGTGTGTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTG CGAGCGCACAGGCAGCCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGGAGC GAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGATGTGAAAGGCTCCATCATAT GTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGTCTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAA CCCAGCATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCTCTCATGACGTCC GCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATCGTAGTGGAGTCAGCCTGGCCAGTGCGTAGTCCCGTCT CTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTTGCTTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTG CACGCCCAGAGGTATTGGGCTCGTCAGATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCT CGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTACCGAACAATTGACTTACCGACATC CTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCCGCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGA CGGCCGCTCATCAGGACCAGACCAGTCTCAATGTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTG

Figure 9 : Séquence nucléotidique du vecteur pELP (suite), contenant le promoteur du gène laccase (4457-6983), un marqueur de résistance à la phléomycine (507-1822) et le terminateur du gène sc3 (71-507)

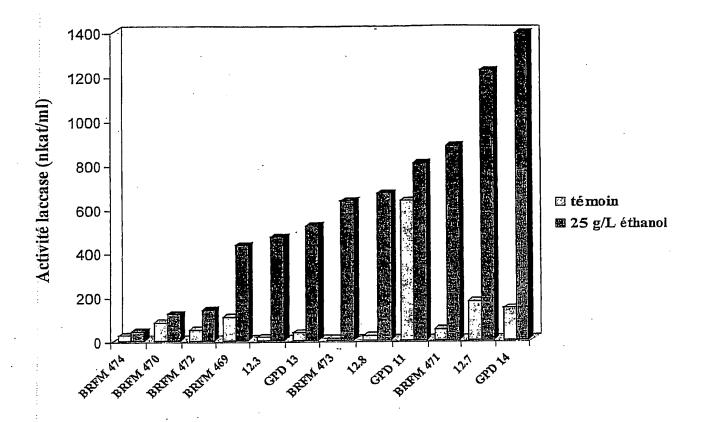
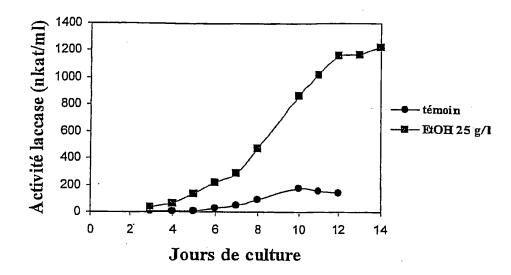


Figure 10 : Résultats de production des transformants présentant les activités les plus importantes. La culture a été effectuée avec ou sans (témoin) éthanol



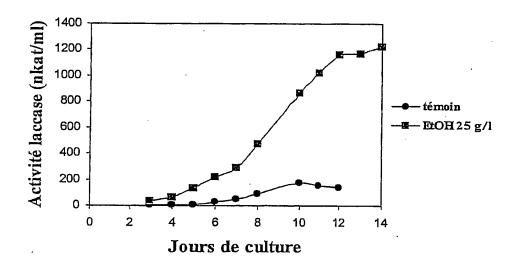


Figure 11 : Suivi des activités laccase des transformants GPD 14 et 12.7 en fonction du temps avec ou (témoin) sans éthanol

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TGGGGGGGTTGGTTCTATATCAAAATGATCTTCTCCCTGGGCTTTCCTCGTCCTTGTTTTCGTCTTGTCAGTGCCGCGACATGCTTTTATTAAACCAT	J.00
TGG CCAGCTTGCCCGCGCC CAAGGAGATA GCATA AT CGCCTGAGAAACCTAGTCGTTCTATGGCCGTTTATCTTTTTTTT	200
TCA GARTATARAGGCCTATTGTGATACGGTTCATCTARCCCCAGCGTCCCCTCCGARGATGGGCTGCCTCTCACTGTTCGCCATTCCTTACTGCTTTARA	300
M G C L S L F A F L T A L N CTCAGTTCATGCCCCTGTGCGTGCTACGGACGTGACGTG	14 400
SVERAVGPVTDLTLVDTVAFDGAAFACTGAAAAACTGAAAAACTGAAAAAACTGAAAAAACTGAAAAAAACTGAAAAAAACTGAAAAAAAA	_43
CGACTGTAAATGCCGGATTTGAGTTTCTAATTATAATCTTCCAGCCATTGTCGTCCAAGAGGAACCAAACTCCGTCATTGGTCCGGTCATCGTAGGTGGG	500
TAIVVOEEPNSVIGPVIV 💎.	60 600
TAGCTACGAGTCTTCCTCCTTCATTTAGCTCATCACCAAGTGATATGATATTAATTA	74.
TTGGATTCTCCGAACATGCGCCAATCTACTTCCATTCGCATGGCATCTTCCAAGGAAACG <u>GTACGT</u> GGTATATCGGATAATCTATCTGTATCCAT	700 95
L D S P N M R Q S T S I H W H G I F Q G N GACTCGARTATAGGTCAGAATTGGGCIGGTCGTTGGCCTTCCTGAAGCCTGCTCGARTTATCTTCCTGAATTTTTAGATGGCGCCGCATTCGTTAACC	800
GONWA DGAAFVN	107
AGGTAAGGAGATGTTCCTGCCTTCGTTTCCCCAGAACTAATTATCCTAGTGCCCCCATTGCCCCCGGAGGGGACTCGTTCTTGTACGACTTTACCGAACCT C P I A P G G D S F L Y D F T E P	900 125
TTCCAGACTGGCACATTTTGGTATCATTCCCATTTATCAACTCAATACTGCGATGGACGGGGGAGCATTCGTCGTTCGT	
FQTGTFWYHSRLSTQYCDGLRGAFV	150
CCGCTTTCTTCTCACTTATCTAGATCTACGATCCGCTCGACCCTTACCGGTTGCTCTACGATGTCGACGACTCGACTACTCTACTCTGGCGACTG	1100
TYDFLDPYRLLYDVDDESTVITLADW	176
GTACCACAGCTATGCGGAGGACATTCTAATCGCGTAGGAGATTTTCCCAAGATGTCTCCTCTCTCT	191
y h s y a e d i l i. a Tecteatgatggteacggargattcgccggagccggacggcaacggcaactatetgteattactgttgagcatggaaagcg <u>ctaggc</u> attctccct	
. T I. T N C H G R F A G A G G T A T E L S V I T V E H G K R	220
CGGCTTTG TAGAT GTGTCTAATTTGTGAT AGCT ACCGATTGCGATTTGCCAAT ATCGCTTGTG ACCGT TGGTTTGCCGTGAAA ATCGATAGCC ATACGAA	1400
Y R L R F A N I A C D P W F A V K I D S H T N	243
CCTTCGCGTTATCGAAGCTGACGGTATTACTACTGTGCCTGTCACGGTGGACTCCTTCAATGTAGGCTTACCCTTAGCACTTTCCCACTCTGGATCCTCT	254
TAT GACTT CCCAA GATCTTTGTGGGCCAACGAT ATAGTGTCAT CCTCCATGCCAACCAGCCTGTTGGAAACTACTGTAAGCTGCCTAAATGTT GCATGAC	1600
T F V G O R Y S V I L H A N Q P V G N Y	274
TGTCCATGATTCTAACCCCGCCAGGGATTCGGGCCGCTCCGAACGGCGTGAGCAATTTCGCGGGTGGGATCGACTCGGCTATTCTCCGTTATGTTGGCGC WIRAAPNGVSNFAGGIDSAILRYVGA	300
CCCAGRAGAAGAGCCCAACACTAGTGAGGATACTCCATCCGACACACTTCAAGAGCAGGATCTTCACCCGCTGATCCTACCCGGCGCGCAAGGCATCCAC	1800
	333
TCCCTTGGGGCCGCCGACGTTGTCCACACCGTATCAATGGAGTTT <u>GTGAGT</u> GTGGCGACTTTTCTGGCCCCCTTTATTAATATAATA	1900
S R G A A D V V H T V S M E F	348
GCAAACTT CCAATTCCTCCTGGATGGCGTGGCCTTCCAGCCGTGCGTCATCTCTTTCAAAGAATTTATCTAGCTGACGATTTTGAAATGTAGCCCGACCA	357
	2100
. M P V T. T. O T T. S G A O T A N T L L P A G S F I Q A S H N D I V E L	391
CAATTTCCCAGCTGTCAACGTAGCCGCTGTCGGTGGACCGTGCGTCCCATCTTTCCTTGCCAGCTTGAAATTTACGCTCTTTTAGACATCCAATCCATCT	2200
THE ACCIC ACCIGGRACCI THE COTTATE GOATATEACT TATTATTAC CATGCCATGCATTC GACGTTATAC GOTOT GOTOG AACGAACTCC CATAACT	
HGHAFDVIRSAGINSDN	420
GGTTCAATCCG <u>GTATTT</u> TCATTCGACTTCCATAAGATGACGATGGCTCACTATGGTTTTTACCCAGCCTCGCAGAGATGTCGTATCCACCGGTACCGGTACCGATC W F N P P R D V V S T G T D	441
CTA AT GRCAAT GT GTACGTGTTT CGCTATTGAT TGTCCGTTTT GATTT GACTGTTGGACAGCACCCATT CGCTT CCGGGCCGACAACCC GT ACGTAAACTG	2500
TIRFRADNP	455
CTGAATCTCTCGTTGTCTTTGGTTCTCATAATCTCATCACAGGTCCATGGTTCCTTCACTGCCACATTGACTGGCACCTTGAACTCGGCTTTGCTTTGGT G P W F L H C H I D W H L E L G F A L V	2600 475
GAT TGCAGAAGCGCCTAGCGAAT GGGACAGCGA CATTAACCCT CCTGGTGCCCTGCCTGCACCTTTTCTCCCCTACACTTGCTAAGATCGCTCTAGCTG	
T N E N D E E H D E D T N P P	491
COT GGGAT GACCTATGCCTACGTTCGCTTGGCTTCTCTTTTACTATTTCAAGTTTCCTCACATTCTCACAGATATGATGCCCTGCCGCCTGAG A W D D L C P T F A W L L F Y Y F R F P H I L N F T D M M P C R L S	2800 525
A W D D L C P T F A W L L F Y Y F K F P H I L N F T D M G F C K L S CAGCAGTA ATCGAGTTA ATCGTTA ATCGTTA TATCTTA TATCGACACATTCACTATTCAA	
e e n e v v n t. n v e	536
CCT ACGGATTTTCCCTCGCACCTGAATTTCGGTGCTAGATCCCCATCCTTCGTGGAGTAGGAAAGAAA	
art ritata cataa-ogt cceteggettag ttaatt cet	3037

Gène de la laccase d'Halocyphina villosa

Figure 12

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